

Amendments to the Specification

Please replace the paragraph beginning at page 14, line 13, with the following rewritten paragraph:

Figure 4 shows the Mt-Lig gene with the principle catalytic domains indicated (primase domain 1-324, nuclease domain 325-447 and ligase domain 448-759). I represents conserved motif: RLVFDLDPGE (SEQ ID NO: 1), II represents SGSKGLHLYT (SEQ ID NO: 2) and III represents KVFVDW (SEQ ID NO: 3). Variants of motif I include RLVFDLDPGE (SEQ ID NO: 72); ELVFDIDMTD (SEQ ID NO: 75); and ELVFDIDMDD (SEQ ID NO: 81). Variants of motif II include SGSKGLHLYT (SEQ ID NO: 73); SGRRGVHCWV (SEQ ID NO: 76); SGRRGIHCWV (SEQ ID NO: 79); SGRRGAHCWV (SEQ ID NO: 82); and SGRRGIHAWI (SEQ ID NO: 84). Variants of motif III include KVFVDW (SEQ ID NO: 74); FPRLDI (SEQ ID NO: 77); FPRLDV (SEQ ID NO: 78); YPRLDI (SEQ ID NO: 80); YPKLDV (SEQ ID NO: 83); and YPRLDV (SEQ ID NO: 85).

Please replace the paragraph beginning at page 14, line 34, with the following rewritten paragraph:

Figure 7 shows a schematic of the interaction of the nuclease, polymerase and ligase activities of Mt-Lig in NHEJ. The sequences shown are CTGCAGATCATGCGCCGGATTGCCCC (SEQ ID NO: 20); GGTACGTGGTTTC (SEQ ID NO: 86); CGGCGCATGATCTGCAG (SEQ ID NO: 87); GAAACCACGTACCGGGGTGT (SEQ ID NO: 88); CTGCAGATCATGCGCCGGATTGCCCCGGTACGTGGTTTC (SEQ ID NO: 89); and GAAACCACGTACCGGGGCAATCCGGCGCATGATCTGCAG (SEQ ID NO: 90).

Please replace the paragraph beginning at page 16, line 20, with the following rewritten paragraph:

Rv0937c (*M. tuberculosis* Ku, 274 amino acids, 30.9 kD) was amplified using 5' primer (5'-ATG CGA GCC ATT TGG ACG GG-3') (SEQ ID NO: 4) and 3' primer (5'- GGA TCC TCA CGG AGG CGT TGG GAC G-3') (SEQ ID NO: 5).

Please replace the paragraph beginning at page 16, line 24, with the following rewritten paragraph:

Rv0938 (*M. tuberculosis* ligase, 759 amino acids, 83.6 kD) was amplified using 5' primer (5'-ATG GGT TCG GCG TCG GAG CA-3') (SEQ ID NO: 6) and 3' primer (5'-TCC TCA TTC GCG CAC CAC CTC ACT GG-3') (SEQ ID NO: 7).

Please replace the paragraph beginning at page 18, line 29, with the following rewritten paragraph:

Equal amounts of the labelled and unlabelled oligonucleotides were annealed by incubation at 70°C for 10min, 50°C for 10 min, 40°C for 10 min, 18°C for 10 min, and then on ice for 5 min, to generate a linear duplex with the desired nucleotide gap using the following pairs of oligonucleotides; 5'-³²P labelled 15-mer (5'-CTGCAGCTGATGCGC-3') (SEQ ID NO: 8) annealed to 20-mer (5'-ATCCGGCGCATCAGCTGCAG-3') (SEQ ID NO: 9); 5'-³²P labelled 15-mer (5'-CTGCAGCT-GATGCGC-3') (SEQ ID NO: 8) annealed to 25-mer (5'-AGTCGATCCTGCGCATCATCTGCAG-3') (SEQ ID NO: 10); 5'-³²P labelled 15-mer (5'-CTGCAGCTGATGCGC-3') (SEQ ID NO: 8) annealed to 41-mer (5'-ACCCGGGGATCCGTACAGTCTATCCGGCGCATCAGCTGCAG-3') (SEQ ID NO: 11).

Please replace the paragraph beginning at page 20, line 3, with the following rewritten paragraph:

Reaction mixtures (10 µl) containing 25 mM Tris-HCl (pH 7.5), 10 mM MgCl₂, 1 mM DTT, 100 µg/ml BSA, 100 nM 5'-³²P labeled 50mer substrate (5'-GTA ACA AAG TTT GGA TTG CTA CTG ACC GCT CTC GTG CTC GTC GCT GCG TT-3') (SEQ ID NO: 12), 3µg Mt-lig, and, as indicated, 50 µM ATP or 50 µM dATP. Reactions were incubated at 25 °C for 2 h and terminated by the addition of 1µl loading buffer. After heat denaturation at 90 °C for 2 min, 4 µl of each reaction was loaded onto a 10% polyacrylamide-8M urea gel. After separation by electrophoresis, labelled products were detected by phosphor-imager analysis.

Please replace the paragraph beginning at page 20, line 26, with the following rewritten paragraph:

Linear duplexes with complementary single strand ends were constructed by annealing the following pairs of oligonucleotides; 5'-³²P labelled 50-mer (5'-GTC TGT CTC ACT ATT AGA ACC CTT TAG AGT CAT GCG TCG CGA GGC AAC GC-3') (SEQ ID NO: 13) annealed to 43-mer (5'-GCC TCG CGA CGC ATG ACT CTA AAG GGT TCT AAT AGT GAG ACA G-3') (SEQ ID NO: 14); 41-mer (5'-GCG ACG AGC ACG AGA GCG GTC AGT AGC AAT CCA AAC TTT GT-3') (SEQ ID NO: 15) annealed to 50-mer (5'-GTA ACA AAG TTT GGA TTG CTA CTG ACC GCT CTC GTG CTC GTC GCT GCG TT-3') (SEQ ID NO: 16). Equal amounts of labelled and unlabeled duplexes (100 nM of each) were incubated with various amounts of Mt-Lig in reaction mixtures (10 µl) containing 25 mM Tris-HCl (pH 7.5), 10 mM MgCl₂, 50µM each of dNTPs and 1 mM ATP at 25 °C for 2 h. Reactions were terminated by the addition of 1µl loading buffer. After heat denaturation at 90 °C for 2 min, 4 µl of each reaction was loaded onto a 10% polyacrylamide-8M urea gel. After separation by electrophoresis, labelled products were detected by phosphor-imager analysis.

Please replace the paragraph beginning at page 21, line 8, with the following rewritten paragraph:

Linear duplexes with complementary single-strand ends were constructed by annealing pairs of oligonucleotides; 5'-³²P labeled 524-mer (5'-CTG TCT GTC TCA CTA TTA GAA CCC TTT AGA GTC ATG CGT CGC GAG GCA ACG C-3') (SEQ ID NO: 17) annealed to 43-mer; 41-mer annealed to 50-mer. 5'-³²P labelled 20-mer (5'-GAAACCACGTACCGGCGTGT-3') (SEQ ID NO: 18) annealed to 13mer (5'-CTTTGGTCGATGG-3') (SEQ ID NO: 19); 26mer (5'-CTGCAGATCATGCGCCGGATTGCCCC-3') (SEQ ID NO: 20) annealed to 17-mer (5'-GACGTCTAGTACGCGGC-3) (SEQ ID NO: 21). Alignment of the complementary single strands generates a ligatable nick in both the unlabelled and labelled strand and a single-nucleotide gap in the labelled strand. A similar strategy was used to construct pairs of duplexes with single-strand extensions that, when aligned, give differently sized gaps with and without single-strand flaps. Equal amounts of the labelled and unlabelled duplexes (100 nM) were incubated with Mt-ligase in 50 mM potassium acetate, 20 mM Tris-acetate, 10 mM magnesium acetate, 1 mM dithiothreitol, pH 7.9 @ 25°C. The reactions were supplemented with 100 µg/ml

BSA and incubated at 37°C for 30 min. The reactions were stopped by the addition of gel loading buffer (95% (v/v) formamide, 0.09% (w/v) bromphenol blue, and 0.09% (w/v) xylene cyanol). After separation by denaturing gel electrophoresis, labelled DNA molecules in the dried gel were detected and quantitated by Phosphor-Imager analysis or x-ray exposure. In assays to measure both DNA synthesis and ligation, the 5' termini of unlabelled oligonucleotides were phosphorylated.

Please replace the paragraph beginning at page 24, line 6, with the following rewritten paragraph:

~10⁸ cells from a 2-day yeast culture in glucose synthetic defined medium lacking uracil and leucine were inoculated into fresh 25 ml of the same medium with galactose as the carbon source. This culture was shaken at 30 C for 2 days, and then diluted back 30-fold into 25 ml fresh medium lacking adenine. Following an additional 2 days shaking, ~6 x 10⁷ cells were harvested and genomic DNA prepared. DNA (0.2 ug, 1.3 x 10⁶ genome equivalents) was then used in a 20 µl PCR reaction with primers OW1708 (5'-HEX-CAAGTATGGATCTCGAGGTT) (SEQ ID NO: 22) and OW1709 (5'-CTGTTCTAGAGGTACCTAGT (SEQ ID NO: 23); 25 cycles of 94 C for 15 seconds and 55 C for 15 seconds). 2 µl was then run on an 8% sequencing gel.

Please replace the paragraph beginning at page 24, line 18, with the following rewritten paragraph:

All colonies analyzed for the nature of their repair event were independently derived. Colonies were purified by streaking and then colony PCR was performed using primers OW603 (5'-CCTTAAGTTGAACGGAGTCC) (SEQ ID NO: 24) and OW620 (5'-CTTGACTAGCGCACTACCAG) (SEQ ID NO: 25), which amplify a 1273 bp fragment surrounding the HO or I-SceI cut sites in successful deletion events (the starting allele is too large to amplify). Recreated I-SceI sites were detected by cleavage *in vitro* with recombinant I-SceI (New England Biolabs) into the expected 574 and 699 bp products. All other individual joint fragments were sequenced with primer OW563 (5'-GGCAGGAGAATTTTCAGCATC) (SEQ ID NO: 26) and their microhomology mediated joining mechanism inferred by comparison with an intact I-SceI or HO cut site.

Please replace Table 2, on page 33, with the rewritten table on the next page of this document:

Bs ykoU:	21-	EVKYDGYR (SEQ ID NO: 27)	-43-	LTLDEIV (SEQ ID NO: 28)	-34-	CFLAFDLLERSG (SEQ ID NO: 29)	-57-	EGIVA (SEQ ID NO: 30)	-15-	WLKYKNFKQAY (SEQ ID NO: 31)	-82-	IGFEFQMDWTE (SEQ ID NO: 32)	-304
Bh 2209:	20-	EVKYDGYR (SEQ ID NO: 33)	-43-	ITIDGELV (SEQ ID NO: 34)	-34-	TLLAFDILELKG (SEQ ID NO: 35)	-57-	EGVVA (SEQ ID NO: 36)	-15-	WLKKNFRQVT (SEQ ID NO: 37)	-81-	HRFRLDVKPAQ (SEQ ID NO: 38)	-306
Mt Lig C:	26-	EPKWDGYR (SEQ ID NO: 39)	-38-	CVIDGEII (SEQ ID NO: 40)	-32-	SFIAPDLLALGD (SEQ ID NO: 41)	-54-	DGVIA (SEQ ID NO: 42)	-13-	MFKIKHLRTAD (SEQ ID NO: 43)	-114-	TAQFNRWRPDR (SEQ ID NO: 44)	-26
Bs yqV:	22-	ELKFDGIR (SEQ ID NO: 45)	-35-	TVLDGEVI (SEQ ID NO: 46)	-26-	VYCVFDVIYKDG (SEQ ID NO: 47)	-47-	EGIVI (SEQ ID NO: 48)	-15-	WLKVINYDYTE (SEQ ID NO: 49)	-81-		
Pa 2138:	235-	ELKLDGYR (SEQ ID NO: 50)	-38-	SWLDGELV (SEQ ID NO: 51)	-35-	LYVLFDPYHEG (SEQ ID NO: 52)	-49-	EGVIG (SEQ ID NO: 53)	-14-	WIKLKCQLRQE (SEQ ID NO: 54)	-111-	AREVTGERPAG (SEQ ID NO: 55)	-313
Mt Rv0938:	478-	EGKWDGYR (SEQ ID NO: 56)	-38-	VVLDGEAV (SEQ ID NO: 57)	-22-	EFWAFDILLYLDG (SEQ ID NO: 58)	-46-	EGVIA (SEQ ID NO: 59)	-15-	WVKDKHWNTQE (SEQ ID NO: 60)	-98-	-SSWRGLRPDK (SEQ ID NO: 61)	-8
Bact ATP Consensus:		s . KhDGhR (SEQ ID NO: 62)		. . hpGEhh (SEQ ID NO: 63)		. h . hFDh s (SEQ ID NO: 64)		Eghhh (SEQ ID NO: 65)		hhK . K			
T7 Lig:	31-	EIKYDGYR (SEQ ID NO: 66)	-48-	FMLDGELM (SEQ ID NO: 67)	-49-	HIKLYAILPL-- (SEQ ID NO: 68)	-62-	EGLIV (SEQ ID NO: 69)	-14-	WWKMKPENEAD (SEQ ID NO: 70)	-96-	PSFVM-PRGTE (SEQ ID NO: 71)	-7
Motif		I		III		IIIa		IV		V		VI	

Table 2

Please amend the specification to enter the formal Sequence Listing submitted herewith.

STATEMENT IN COMPLIANCE WITH 37 C.F.R. § 1.821(f)

In compliance with 37 C.F.R. § 1.821(f), the undersigned declares that the nucleotide and/or amino acid sequences presented in the paper copy of the "Sequence Listing" submitted herewith are the same as the sequences contained in the computer-readable form of the "Sequence Listing."